Amendments to Specification

Please replace the paragraph at page 13, lines 16-24, with the following:

SEQ ID NO:5 shows the 5' to 3' nucleotide sequence of 1462 ~~1369~~ base pairs of soybean (*Glycine max* ~~Glycine max~~) cDNA which encodes microsomal delta-12 desaturase in plasmid pSF2-169K. Nucleotides 108 to 110 and nucleotides 1245 to 1247 are, respectively, the putative initiation codon and the termination codon of the open reading frame (nucleotides 108 to 1247). Nucleotides 1 to 107 and 1248 to 1462 ~~1369~~ are, respectively, the 5' and 3' untranslated nucleotides.

Please replace the paragraph at page 13, lines 25-27 with the following:

SEQ ID NO:6 is the 379 ~~381~~ amino acid protein sequence deduced from the open reading frame (nucleotides 108 to 1247 ~~113 to 1258~~) in SEQ ID NO:5.

Please replace the paragraph at page 14, lines 11-13, with the following:

SEQ ID NO:10 is the 224 ~~219~~ amino acid protein sequence deduced from the open reading frame (nucleotides 1 to 673 ~~657~~) in SEQ ID NO:9.

Please replace the paragraph at page 14, lines 14-23, with the following:

SEQ ID NO:11 shows the 5' to 3' nucleotide sequence of 1369 base pairs of castor (*Ricinus communis* ~~Ricinus communis~~) cDNA which encodes part of a microsomal delta-12 desaturase or desaturase-related enzyme in plasmid pRF197C-42. Nucleotides 184 to 186 and nucleotides 1345 to 1347 ~~1340 to 1342~~ are, respectively, the putative initiation codon and the termination codon of the open reading frame (nucleotides 184 to 1347). Nucleotides 1 to 183 and 1348 to 1369 are, respectively, the 5' and 3' untranslated nucleotides.

Please replace the paragraph at page 14, lines 24-26, with the following:

SEQ ID NO:12 is the 387 amino acid protein sequence deduced from the open reading frame (nucleotides 184 to 1347 ~~1342~~) in SEQ ID NO:11.

Please replace the paragraph beginning at page 38, line 7, and continuing through page 39, line 13, with the following:

Corn microsomal delta-12 desaturase cDNA was isolated using a PCR approach. For this, a cDNA library was made to poly A^+ RNA from developing corn embryos in Lambda Zap II vector. This library was used as template for PCR using sets of degenerate oligomers NS3 (SEQ ID NO:13) and RB5A/B (SEQ ID NOS:16 and 17) as sense and antisense primers, respectively. NS3 and RB5A/B correspond to stretches of amino acids 101-109 and 318-326, respectively, of SEQ ID NO:2, which are conserved in most microsomal delta-12 desaturases (for example, SEQ ID

NOS:2, 4, 6, 8). PCR was carried out using a PCR kit (Perkin-Elmer) by 40 cycles of 94°C 1', 45°C, 1 min, and 55°C, 2 min. Analyses of the PCR products on an agarose gel showed the presence of a product of the expected size (720 bp), which was absent in control reactions containing either the sense or antisense primers alone. The fragment was gel purified and then used as a probe for screening the corn cDNA library at 60°C as described above. One positively-hybridizing plaque was purified and partial sequence determination of its cDNA showed it to be a nucleotide sequence encoding microsomal delta-12 desaturase but truncated at the 3' end. The cDNA insert encoding the partial desaturase was gel isolated and used to probe the corn cDNA library again. Several positive plaques were recovered and characterized. DNA sequence analysis revealed that all of these clones seem to represent the same sequence with the different length of 5' or 3' ends. The clone containing the longest insert, designated pFad2#1, was sequenced completely. The total length of the cDNA is 1790 bp (SEQ ID NO:7) comprising of an open reading frame from nucleotide 165 to 1328 bp that encoded a polypeptide of 387 ~~388~~ amino acids. The deduced amino acid sequence of the polypeptide (SEQ ID NO:8) shared overall identities of 71%, 40%, and 38% to *Arabidopsis* ~~Arabidopsis~~ microsomal delta-12 desaturase, *Arabidopsis* ~~Arabidopsis~~ microsomal delta-15 desaturase, and *Arabidopsis* ~~Arabidopsis~~ plastid delta-15 desaturase, respectively. Furthermore, it lacked an N-terminal amino acid extension that would indicate it is a plastid enzyme. Based on these considerations, it is concluded that it encodes a microsomal delta-12 desaturase.

Please replace the paragraph beginning on page 39, line 17, and continuing through page 40, line 12, with the following:

Polysomal mRNA was isolated from castor beans of stages I-II (5-10 DAP) and also from castor beans of stages IV-V (20-25 DAP). Ten ng of each mRNA was used for separate RT-PCR reactions, using the Perkin-Elmer RT-PCR kit. The reverse transcriptase reaction was primed with random hexamers and the PCR reaction with degenerate delta-12 desaturase primers NS3 and NS9 (SEQ ID NOS:13 and 14). The annealing-extension temperature of the PCR reaction was 50°C. A DNA fragment of approx. 700 bp was amplified from both stage I-II and stage IV-V mRNA. The amplified DNA fragment from one of the reactions was gel purified and cloned into a pGEM-T vector using the Promega pGEM-T PCR cloning kit to create the plasmid pRF2-1C. The 700 bp insert in pRF2-1C was sequenced, as described above, and the resulting DNA sequence is shown in SEQ ID NO:9. The DNA sequence in SEQ ID NO:9 contains an open-reading frame encoding 219 amino acids (SEQ ID NO:10) which has 81% identity (90% similarity) with amino acids 135

to 353 of the *Arabidopsis* ~~Arabidopsis~~ microsomal delta-12 desaturase described in SEQ ID NO:2. The cDNA insert in pRF2-1C is therefore a 673 ~~676~~ bp fragment of a full-length cDNA encoding a castor bean seed microsomal delta-12 desaturase. The full length castor bean seed microsomal delta-12 desaturase cDNA may isolated by screening a castor seed cDNA library, at 60°C, with the labeled insert of pRF2-1C as described in the example above. The insert in pRF2-1C may also be used to screen castor bean libraries at lower temperatures to isolate delta-12 desaturase-related sequences, such as the delta-12 hydroxylase.

Please replace the paragraph beginning at page 77, line 22, and continuing through pages 78 and 79, with the following:

Corn microsomal delta-12 desaturase cDNA was isolated using a PCR approach. For this, a cDNA library was made to poly A^+ RNA from developing corn embryos in Lambda ZAP II vector (Stratagene). 5-10 ul of this library was used as a template for PCR using 100 pmol each of two sets of degenerate oligomers NS3 (SEQ ID NO:13) and equimolar amounts of RB5a/b (that is, equimolar amounts of SEQ ID NOS:16/17) as sense and antisense primers, respectively. NS3 and RB5a/b correspond to stretches of amino acids 101-109 and 318-326, respectively, of SEQ ID NO:2, which are conserved in most microsomal delta-12 desaturases (SEQ ID NOS:2, 4, 6, 8). PCR was carried out using the PCR kit (Perkin-Elmer) using 40 cycles of 94°C 1 min, 45°C, 1 min, and 55°C, 2 min. Analyses of the PCR products on an agarose gel showed the presence of a product of the expected size (720 bp), which was absent in control reactions containing either the sense or antisense primers alone. The PCR product fragment was gel purified and then used as a probe for screening the same corn cDNA library at 60°C as described above. One positively-hybridizing plaque was purified and partial sequence determination of its cDNA showed it to be a nucleotide sequence encoding microsomal delta-12 desaturase but truncated at the 3' end. The cDNA insert encoding the partial desaturase was gel isolated and used to probe the corn cDNA library again. Several positive plaques were recovered and characterized. DNA sequence analysis revealed that all of these clones seem to represent the same sequence with the different length of 5' or 3' ends. The clone containing the longest insert, designated pFad2#1, was sequenced completely. SEQ ID NO:7 shows the 5' to 3' nucleotide sequence of 1790 base pairs of corn (*Zea mays* ~~Zea mays~~) cDNA which encodes microsomal delta-12 desaturase in plasmid pFad2#1. Nucleotides 165 to 167 and nucleotides 1326 to 1328 are, respectively, the putative initiation codon and the termination codon of the open reading frame (nucleotides 165 ~~164~~ to 1328). SEQ ID

NO:8 is the 387 amino acid protein sequence deduced from the open reading frame (nucleotides 165 ~~164~~ to 1328) in SEQ ID NO:7. The deduced amino acid sequence of the polypeptide shared overall identities of 71%, 40%, and 38% to *Arabidopsis* ~~Arabidopsis~~ microsomal delta-12 desaturase, *Arabidopsis* ~~Arabidopsis~~ microsomal delta-15 desaturase, and *Arabidopsis* ~~Arabidopsis~~ plastid delta-15 desaturase, respectively. Furthermore, it lacked an N-terminal amino acid extension that would indicate it is a plastid enzyme. Based on these considerations, it is concluded that it encodes a microsomal delta-12 desaturase.

Please note: In Table 7, underlining below upper case letters designates conserved amino acid residues; this underlining was present in the original Table 7 and does not represent newly added text. For the purpose of this table only, new text is indicated by double underlining. Please replace Table 7, beginning on page 47, and continuing through page 48, with the following:

TABLE 7

Amino Acid Sequences Conserved Between
Plant Microsomal Delta-12 Desaturases and Microsomal and
Plastid Delta-15 Desaturases

Region	Conserved AA Positions in SEQ ID NO:2	Consensus Conserved AA Sequence in Δ^{12}Desaturases	Consensus Conserved AA Sequence in Δ^{15}Desaturases	Consensus AA Sequence
A	39-44	AIPPHC (SEQ ID NO:18)	AIPKHC (SEQ ID NO:19)	AIP(P/K)HC (SEQ ID NO:20)
B	86-90	WP(L/I)YW (SEQ ID NO:21)	WPLYW (SEQ ID NO:22)	WP(L/I)YW (SEQ ID NO:23)
C	104-109	AHECGH (SEQ ID NO:24)	GHDCGH (SEQ ID NO:25)	(A/G)H(D/E)CGH (SEQ ID NO:26)
D	130-134	LLVPY (SEQ ID NO:27)	ILVPY (SEQ ID NO:28)	(L/I)LVPY (SEQ ID NO:29)
E	137-142	WKYSHR (SEQ ID NO:30)	WRISHR (SEQ ID NO:31)	W(K/R)(Y/I)SHR (SEQ ID NO:32)
F	140-145	SHRRHH (SEQ ID NO:33)	SHRTHH (SEQ ID NO:34)	SHR(R/T)HH (SEQ ID NO:35)
G	269-274	ITYLQ (SEQ ID NO:36)	VTYLH (SEQ ID NO:37)	(I/V)TYL(Q/H) (SEQ ID NO:38)

H	279-282	LPHY (SEQ ID NO:39)	LPWY (SEQ ID NO:40)	LP(H/W)Y (SEQ ID NO:41)
I	289-294	WL(R/K)GAL (SEQ ID NO:42)	YLRGGL (SEQ ID NO:43)	(W/Y)L(R/K)G(A/G)L (SEQ ID NO:44)
J	296-302	TVDRDYG (SEQ ID NO:45)	TLDRDYG (SEQ ID NO:46)	T(V/L)DRDYG (SEQ ID NO:47)
K	314-321	THVAHHLF (SEQ ID NO:48)	THVIHHLF (SEQ ID NO:49)	THV(A/I)HHLF (SEQ ID NO:50)
L	318-327	HHLFSTMPHY (SEQ ID NO:51)	HHLFPQIPHY (SEQ ID NO:52)	HHLF(S/P)(T/Q)(I/M)PHY ~~HHFL(S/P)(T/Q)(I/M)PHY~~ (SEQ ID NO:53)

Please replace the paragraph beginning on page 85, line 31, continuing through page 86 and onto page 87, with the following:

The pML70 vector contains the KTi3 promoter and the KTi3 3' untranslated region and was derived from the commercially available vector pTZ18R (Pharmacia) via the intermediate plasmids pML51, pML55, pML64 and pML65. A 2.4 kb Bst BI/Eco RI fragment of the complete soybean KTi3 gene (Jofuku and Goldberg (1989) Plant Cell 1:1079-1093), which contains all 2039 nucleotides of the 5' untranslated region and 390 bases of the coding sequence of the KTi3 gene ending at the Eco RI site corresponding to bases 755 to 761 of the sequence described in Jofuku et al (1989) Plant Cell 1:427-435, was ligated into the Acc I/Eco RI sites of pTZ18R to create the plasmid pML51. The plasmid pML51 was cut with Nco I, filled in using Klenow, and religated, to destroy an Nco I site in the middle of the 5' untranslated region of the KTi3 insert, resulting in the plasmid pML55. The plasmid pML55 was partially digested with Xmn I/Eco RI to release a 0.42 kb fragment, corresponding to bases 732 to 755 of the above cited sequence, which was discarded. A synthetic Xmn I/Eco RI linker containing an Nco I site, was constructed by making a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for an Xmn I site (5'-TCTTCC-3') and an Nco I site (5'-CCATGGG-3') followed directly by part of an Eco RI site (5'-GAAGG-3'). The Xmn I and Nco I/Eco RI sites were linked by a short intervening sequence (5'-ATAGCCCCCCAA-3'; SEQ ID NO:54). This synthetic linker was ligated into the Xmn I/Eco RI sites of the 4.94 kb fragment to create the plasmid pML64. The 3' untranslated region of the KTi3 gene was amplified from the sequence described in Jofuku et al (Ibid.) by standard PCR protocols (Perkin Elmer Cetus, GeneAmp PCR kit) using the primers ML51 and

ML52. Primer ML51 contained the 20 nucleotides corresponding to bases 1072 to 1091 of the above cited sequence with the addition of nucleotides corresponding to Eco RV (5-'GATATC-3'), Nco I (5'-CCATGG-3'), Xba I (5'-TCTAGA-3'), Sma I (5'-CCCGGG-3') and Kpn I (5'-GGTACC-3') sites at the 5' end of the primer. Primer ML52 contained to the exact compliment of the nucleotides corresponding to bases 1242 to 1259 of the above cited sequence with the addition of nucleotides corresponding to Sma I (5'-CCCGGG-3'), Eco RI (5'-GAATTC-3'), Bam HI (5'-GGATCC-3') and Sal I (5'-GTCGAC-3') sites at the 5' end of the primer. The PCR-amplified 3' end of the KTi3 gene was ligated into the Nco I/Eco RI sites of pML64 to create the plasmid pML65. A synthetic multiple cloning site linker was constructed by making a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for Pst I (5'-CTGCA-3'), Sal I (5'-GTCGAC-3'), Bam HI (5'-GGATCC-3') and Pst I (5'-CTGCA-3') sites. The linker was ligated into the Pst I site (directly 5' to the KTi3 promoter region) of pML65 to create the plasmid pML70.

Please replace the paragraph beginning at page 88, line 13, and continuing onto page 89 of the specification, with the following:

The pCW108 vector contains the bean phaseolin promoter and 3' untranslated region and was derived from the commercially available pUC18 plasmid (Gibco-BRL) via plasmids AS3 and pCW104. Plasmid AS3 contains 495 base pairs of the bean (Phaseolus vulgaris) phaseolin (7S seed storage protein) promoter starting with 5'-TGGTCTTTTGGT-3' (SEQ ID NO:55) followed by the entire 1175 base pairs of the 3' untranslated region of the same gene (see sequence descriptions in Doyle et al., (1986) J. Biol. Chem. 261:9228-9238 and Slightom et al.,(1983) Proc. Natl. Acad. Sci. USA, 80:1897-1901. Further sequence description may be found in WO 9113993) cloned into the Hind III site of pUC18. The additional cloning sites of the pUC18 multiple cloning region (Eco RI, Sph I, Pst I and Sal I) were removed by digesting with Eco RI and Sal I, filling in the ends with Klenow and religating to yield the plasmid pCW104. A new multiple cloning site was created between the 495bp of the 5' phaseolin and the 1175bp of the 3' phaseolin by inserting a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for a Nco I site (5'-CCATGG-3') followed by three filler bases (5'-TAG-3'), the coding sequence for a Sma I site (5'-CCCGGG-3'), the last three bases of a Kpn I site (5'-TAC-3'), a cytosine and the coding sequence for an Xba I site (5'-TCTAGA-3') to create the plasmid pCW108. This plasmid contains unique Nco I, Sma I, Kpn I and Xba I sites directly behind the phaseolin promoter. The 1.4 kb Eco RV/Sma I fragment from pSF2-169K was ligated into the Sma I site of the commercially

available phagemid pBC SK+ (Stratagene). A phagemid with the cDNA in the desired orientation was selected by digesting with Pfl MI/Xho I to yield fragments of approx. 1 kb and 4 kb and designated pM1-SF2. The 1.4 kb Xmn I/Xba I fragment from pM1-SF2 was inserted into the Sma I/Xba I sites of pCW108 to yield the plasmid pBS11, which has the soybean delta-12 desaturase cDNA in the reverse (3'-5') orientation behind the phaseolin promoter. The plasmid pBS11 was digested with Bam HI and a 3.07 kb fragment, representing the phaseolin promoter/antisense desaturase cDNA/phaseolin 3' end transcriptional unit was isolated by agarose gel electrophoresis and ligated into the Hind III site of pML18 (described above). When the resulting plasmids were digested with Xba I, plasmids containing inserts in the desired orientation yielded 2 fragments of 8.01 and 1.18 kb. A plasmid with the transcriptional unit in the correct orientation was selected and was designated pBS14.

Please replace the paragraph at page 112, lines 7-21 of the specification, with the following:

An extended poly A tail was removed from the canola delta-12 desaturase sequence contained in plasmid pCF2-165D and additional restriction sites for cloning were introduced as follows. A PCR primer was synthesized corresponding to bases 354 through 371 of SEQ ID NO:3. The second PCR primer was synthesized as the complement to bases 1253 through 1231 with 15 additional bases (GCAGATATCGCGGCC; SEQ ID NO:56) added to the 5' end. The additional ~~additonal~~ bases encode both an EcoRV site and a NotI site. pCF2-165D was used as the template for PCR amplification using these primers. The 914 base pair product of PCR amplification was digested with EcoRV and PflMI to give an 812 base pair product corresponding to bases 450 through 1253 of pCF2-165D with the added NotI site.

Please replace the paragraph beginning on page 114, line 3, and continuing onto page 115 of the specification, with the following:

Canola napin promoter expression cassettes were constructed ~~consturcted~~ as follows: Ten oligonucleotide primers were synthesized based upon the nucleotide sequence of napin lambda clone CGN1-2 published in European Patent Application EP 255378). The oligonucleotide sequences were:

- BR42 and BR43 corresponding to bases 1132 to 1156 (BR42) and the complement of bases 2248 to 2271 (BR43) of the sequence listed in Figure 2 of EP 255378.

- BR45 and BR46 corresponding to bases 1150 to 1170 (BR46) and the complement of bases 2120 to 2155 (BR45) of the sequence listed in Figure 2 of EP 255378. In addition BR46 had bases corresponding to a Sal I site (5'-GTCGAC-3') and a few additional bases (5'-TCAGGCCT-3') at its 5' end and BR45 had bases corresponding to a Bgl II site (5'-AGATCT-3') and two (5'-CT-3') additional bases at the 5' end of the primer,
- BR47 and BR48 corresponding to bases 2705 to 2723 (BR47) and bases 2643 to 2666 (BR48) of the sequence listed in Figure 2 of EP 255378. In addition BR47 had two (5'-CT-3') additional bases at the 5' end of the primer followed by bases corresponding to a Bgl II site (5'-AGATCT-3') followed by a few additional bases (5'-TCAGGCCT-3'),
- BR49 and BR50 corresponding to the complement of bases 3877 to 3897 (BR49) and the complement of bases 3985 to 3919 (BR50) of the sequence listed in Figure 2 of EP 255378. In addition BR49 had bases corresponding to a Sal I site (5'-GTCGAC-3') and a few additional bases (5'-TCAGGCCT-3') at its 5' end,
- BR57 and BR58 corresponding to the complement of bases 3875 to 3888 (BR57) and bases 2700 to 2714 (BR58) of the sequence listed in Figure 2 of EP 255378. In addition the 5' end of BR57 had some extra bases (5'-CCATGG-3') followed by bases corresponding to a Sac I site (5'-GAGCTC-3') followed by more additional bases (5'-GTCGACGAGG-3'; SEQ ID NO:57). The 5' end of BR58 had additional bases (5'-GAGCTC-3') followed by bases corresponding to a Nco I site (5'-CCATGG-3') followed by additional bases (5'-AGATCTGGTACC-3'; SEQ ID NO:58).
- BR61 and BR62 corresponding to bases 1846 to 1865 (BR61) and bases 2094 to 2114 (BR62) of the sequence listed in Figure 2 of EP 255378. In addition the 5' end of BR 62 had additional bases (5'-GACA-3') followed by bases corresponding to a Bgl II site (5'-AGATCT-3') followed by a few additional bases (5'-GCGGCCGC-3').